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**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/594,188 | KOLOSSOV ET AL. | |
| | Examiner | Art Unit | |
| | Shin-Lin Chen | 1632 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *17 November 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-44* is/are pending in the application.

4a) Of the above claim(s) *34-44* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-33* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *25 September 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *5-6-08 & 8-1-08*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

## DETAILED ACTION

### *Election/Restrictions*

1. Applicant's election with traverse of group X, claims 1-33, in the reply filed on 11-17-08 is acknowledged. The traversal is on the ground(s) that the special technical feature common to groups I-XXI is the use of agitation techniques for culturing multi- or pluripotent cells for the generation of cell aggregates in high density and high quality. The rocking table taught by '711 patent is not for generation of aggregates themselves, but rather only to prevent the already existing aggregates from stinking to the surface during culture. The '678 patent does not discuss the use of agitation as a way of generating cell aggregates in high density and high quality. Further, the type of cell that the embryoid bodies are differentiated into is the only difference between groups I-X, and there is no serious burden for Examiner to search all those groups. This is not found persuasive because the use of agitation techniques for culturing multi- or pluripotent cells for the generation of cell aggregates is not the common special technical feature for group I-XXI. The special technical feature common to groups XI-XXI is the embryoid body rather than the use of agitation techniques for culturing multi- or pluripotent cells for the generation of cell aggregates. Group XI is drawn to a method for identifying and/or obtaining a drug or for determining the toxicity of a compound by using the embryoid body, and group XII-XXI are basically drawn to a pharmaceutical composition comprising the embryoid body, a kit and a device or apparatus. The putative special technical feature shared by groups I-XXI is a method for producing embryoid body (EB) from multi- or pluripotent cells, or the embryoid body, or cells or tissue produced from said embryoid body, and the putative special technical feature is taught by '711 patent and '678 publication. It should be noted that '711 does teach culturing ES

cell suspension **by gentle and continuous rocking on a rocker for further EBs formation** (column 4, lines 20-31). Further, different cell types that the same or different embryoid bodies differentiated into require different culturing conditions and microenvironments, e.g. different stimulating or inhibiting factors. They have different modes of function and effect, and require separate consideration and search. Thus, there would be serious burden for Examiner to consider all of group I-XXI.

The requirement is still deemed proper and is therefore made FINAL.

2. Claims 34-44 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 11-17-08.

Claims 1-44 are pending. Claims 1-33, and a method for producing embryoid bodies (EBs) from multi- or pluripotent cells with or without mouse fibroblast feeder cells, an embryoid body obtained by said method, and a differentiated cell or tissue derived from the EB, wherein the cells are cardiomyocytes and are genetically engineered, are considered.

### *Claim Objections*

3. Claims 4-33 are objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim cannot depend from any other multiple dependent claim. See MPEP § 608.01(n). Accordingly, the claims have not been further treated on the merits.

### *Claim Rejections - 35 USC § 112*

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 1-33 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The phrase "optionally diluting the suspension" in claim 1 is vague and renders the claim indefinite. The term "optionally" is not a definite term. It is unclear whether "diluting the suspension" is intended in the claim or not. Claims 2-33 depend from claim 1 but fail to clarify the indefiniteness.

The phrase "the method of any one of claims 1 to 3" in claim 4 is vague and renders the claim indefinite. Claim 3 is a multiple dependent claim that depends from claim 1 or 2, and claim 4 depends from claim 3. It is improper that a multiple dependent claim depends from another multiple dependent claim. It is unclear whether claim 4 depends from claim 1 or 2 or both. Similarly, claims 5-33 are all improper multiple dependent claims for the same reasons.

Generally, a multiple dependent claim is a dependent claim which refers back in the alternative to more than one preceding independent or dependent claim. The second paragraph of 35 U.S.C. 112 has been revised in view of the multiple dependent claim practice introduced by the Patent Cooperation Treaty. Thus 35 U.S.C. 112 authorizes multiple dependent claims in applications filed on and after January 24, 1978, as long as they are in the alternative form (e.g., "A machine according to claims 3 or 4, further comprising ---"). Cumulative claiming (e.g., "A machine according to claims 3 and 4, further comprising ---") is not permitted. A multiple dependent claim may refer in the alternative to only one set of claims. A claim such as "A device as in claims 1, 2, 3, or 4, made by a process of claims 5, 6, 7, or 8" is improper. 35 U.S.C. 112 allows reference to only a particular claim. Furthermore, a multiple dependent claim may not serve as a basis for any other multiple dependent claim, either directly or indirectly. These limitations help to avoid undue confusion in determining how many prior claims are actually referred to in a multiple dependent claim. (37 C.F.R. 1.75).

The phrase "wherein said multi- or pluripotent cells are embryonic stem (ES) cells" in claim 3 is vague and renders the claim indefinite. It is well known in the art that embryonic stem cells are pluripotent cells but NOT multipotent cells. It is unclear how a multipotent cell can be an embryonic stem cell.

The phrase "the cells are derived from a murine ES cell line" in claim 4 is vague and renders the claim indefinite. The phrase "derived from" could mean physically, chemically, or genetically modified or no modification. It is unclear what kind of modification and to what extent of the modification or no modification is considered "derived from".

The term "and/or" in claims 5, 6, 19, 21 and 29 is vague and renders the claims indefinite. It is unclear what is intended, "and" or "or" or both at the same time. Changing the term "and/or" to "… or… or both" would be remedial.

The terms "IMDM" and "FCS" in claim 5 is vague and renders the claim indefinite. The terms "IMDM" and "FCS" are abbreviations that can stand for various meanings. It is unclear what meaning is intended in the claim. Spelling out the terms "IMDM" and "FCS" would be remedial.

The phrase "wherein the final concentration of EBs in the suspension culture is about 500/ml" in claim 12 is vague and renders the claim indefinite. It is unclear what unit of "500" is, cell, particle, mg or gram etc.

The phrase "substantially the same" in claim 25 is vague and renders the claim indefinite. It is unclear to what extent is considered "substantially the same". The specification fails to specifically define the phrase "substantially the same".

The phrase "selected from different color versions of enhanced green fluorescent protein (EGFP)" in claim 26 is vague and renders the claim indefinite. It is unclear as to the metes and bounds of what would be considered "different color versions of enhanced green fluorescent protein". The type and boundary of "different color versions of EGFP" is vague and uncertain. It is unclear how many and what color versions of EGFP is intended in the claim.

The terms "alphaMHC" and "MLC2v" in claim 30 is vague and renders the claim indefinite. The terms "alphaMHC" and "MLC2v" are abbreviations that can stand for various meanings. It is unclear what meaning is intended in the claim. Spelling out the terms "alphaMHC" and "MLC2v" would be remedial.

The phrase "derived from" in claim 32 is vague and renders the claim indefinite. The phrase "derived from" could mean physically, chemically, or genetically modified or no modification. It is unclear what kind of modification and to what extent of the modification or no modification is considered "derived from".

***Claim Rejections - 35 USC § 112***

6. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

7. Claims 1-33 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for producing embryoid bodies (EBs) from pluripotent embryonic stem (ES) cells or embryonic germ (EG) cells, does not reasonably provide enablement for a method for producing embyroid bodies (EBs) from multipotent cells, including hematopoietic stem cells, neural stem cells, pancreatic stem cells, follicular stem cells, and any other adult stem cells or progenitor cells, and the production of a differentiated cell which is a cardiomyocyte from said EBs. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

While determining whether a specification is enabling, one considered whether the claimed invention provides sufficient guidance to make and use the claimed invention, if not, whether an artisan would have required undue experimentation to make and use the claimed invention and whether working examples have been provided. When determining whether a specification meets the enablement requirement, some of the factors that need to be analyzed are: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, and whether the quantity of any necessary experimentation to make or use the invention based on the content of the disclosure is "undue" (In re Wands, 858 F.2d at 737, 8 USPQ2d 1400, 1404 (Fed. Cir.1988)).

Furthermore, the USPTO does not have laboratory facilities to test if an invention with function as claimed when working examples are not disclosed in the specification, therefore, enablement issues are raises and discussed based on the state of knowledge pertinent to an art at the time of the invention, therefore skepticism raised in the enablement rejections are those raised in the art by artisans of expertise.

Claims 1-33 are directed to a method for producing embryoid bodies (EBs) from multi- or pluripotent cells, such as embryonic stem cells, comprising agitation of a liquid suspensiion culture of multi- or pluripotent cells in a container until generation of cell aggregates and optionally diluting the suspension and further agitation of the suspension until formation of EBs, wherein the cells could be cultured on embryonic mouse fibroblasts feeder cells before agitation of suspension culture, an embryoid body obtained from said method, and a differentiated cell, which is a cardiomyocyte, or tissue derived from the embryoid body. Claims 5 and 6 specify the

culture medium and conditions. Claim 7 specifies the concentration of multi- or pluripotent cells is about $1x10^6$ to $5x10^6$ cells/ml. Claims 8-10 specify the suspension is cultured for about 6 hours, 16 to 20 hours and in T25 flasks, respectively. Claims 11 and 12 specify the dilution is 1:10 and the final concentration of EBs in the suspension culture is about 500/ml, respectively. Claims 14 and 15 specify the culture of multi- or pluripotent cells has a concentration of $0.1x10^6$ to $0.5x10^6$ cells/ml and the suspension is cultured for about 48 hours, repectively. Claim 16 specifies the EBs are diluted to about 100-2000 EBs/10ml. Claims 17 and 18 specify the cells are differentiated into cardipmyocytes. Claims 19-33 specify the cells are genetically engineered using a selectable marker, such as puromycin resistant gene, and/or a reporter gene, such as EGFP, under the control of a cell type-specific regulatory sequence. Claims 27 and 28 specify the marker gene and reporter gene are contained on the same recombinant nucleic acid molecule and on the same cistron, respectively. Claims 29 and 30 specify the cell type-specific regulatory sequence is atrial- and/or ventricular-specific and is selected from promoters of alphaMHC or MLC2v, respectively.

The specification discloses transfection of mouse embryonic stem cells with palphaMHC-GFP vector comprising GFP gene under the control of the cardiac alpha-myosin heavy chain (alpha-MHC) promoter. The ES cells were cultured on 10 cm Petri dishes in the presence of 15% FCS and $1x10^3$ U/ml LIF on a layer of feeder cells (inactivated mouse embryonic fibroblasts). The ES cells were trypsinized, centrifuged, and resuspended in IMDM medium with 20% FBS, and the ES cells were cultured in suspension at a density of $2x10^6$ cells/ml in a 6 cm Petri dish in IMDM with 20% FCS on a rocking table with or without agitation. EBs in suspension was transferred to a COPAS select particle sorter and the EBs were cultured in

IMDM and 20% FCS, and fluorescent areas representing cardiac cells were detected by fluorescent microscopy (e.g. Examples 1 and 2). The claims encompass producing EBs from pluripotent cells and numerous different multipotent cells, including hematopoietic stem cells, neural stem cells, pancreatic stem cells, follicular stem cells, and any other adult stem cells or progenitor cells.

The specification fails to provide adequate guidance and evidence for how to produce EBs from numerous different multipotent cells, including hematopoietic stem cells, neural stem cells, pancreatic stem cells, follicular stem cells, and any other adult stem cells or progenitor cells.

A search of the state of the art of generating EBs, it is apparent that only pluripotent embryonic stem cells or embryonic germ cells can produce EBs under a certain culturing condition. Kurosawa, H., 2007 (Journal of Bioscience and Bioengineering, Vol. 5, p. 389-398) reports that embryonic stem (ES) cells cultured in suspension in the absence of LIF or MEF feeder layers would spontaneously differentiate and form three-dimensional multicellular aggregates called embryoid bodies (EBs). "EBs recapitulate many aspects of cell differentiation during early embryogenesis, and play an important role in the differentiation of ES cells into a variety of cell types in vitro" (e.g. abstract, p. 389, left column). "EB formation has been utilized widely as a trigger of in vitro differentiation of both mouse and human ES cells" (e.g. p. 389, right column). No literature has reported formation of EBs from various multipotent cells, such as hematopoietic stem cells, pancreatic stem cells, follicular stem cells, neural stem cells, or any other type of adult stem cells or progenitor cells. There is no evidence of record that demonstrate the formation of EBs from any multipotent cells in vitro or in vivo. Absent specific guidance,

one skilled in the art at the time of the invention would not know how to form EBs from numerous different multipotent cells in vitro or in vivo.

For the reasons set forth above, one skilled in the art at the time of the invention would have to engage in undue experimentation to practice over the full scope of the invention claimed. This is particularly true based upon the nature of the claimed invention, the state of the art, the unpredictability found in the art, the teaching and working examples provided, the level of skill which is high, the amount of experimentation required, and the breadth of the claims.

***Claim Rejections - 35 USC § 102***

8. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

9. Claims 1-3 and 31 are rejected under 35 U.S.C. 102(e) as being anticipated by Thomson et al., 2003 (US Patent No. 6,602,711 B1).

Claims 1-3 and 31 are directed to a method for producing embryoid bodies (EBs) from multi- or pluripotent cells, such as embryonic stem cells, comprising agitation of a liquid suspensiion culture of multi- or pluripotent cells in a container until generation of cell aggregates and optionally diluting the suspension and further agitation of the suspension until formation of

EBs, wherein the cells could be cultured on embryonic mouse fibroblasts feeder cells before agitation of suspension culture, and an embryoid body obtained from said method.

Thomson teaches a method for producing primate embryoid bodies from colonies of primate embryonic stem cells by removing the adhering colonies of the embryonic stem cells from the substrate in clumps and then incubating the clumps in a container under conditions that essentially inhibit the clumps from attaching to the container and under conditions in which the clumps of embryonic stem cells coalesce into embryoid bodies (e.g. claim 1). Thomson teaches culturing primate embryonic stem cells, such as rhesus or human ES cells, on inactivated mouse embryonic fibroblasts with culture medium DMED and 20% FBS and the ES cell colonies are removed from the tissue culture plate using physical or chemical methods, such as dispase or collagenase (e.g. column 3, lines 48-67). Thomson further teaches "[o]nce colonies are removed from the tissue culture plate, the ES cells should remain in suspension during further embyoid body formation. This can be achieved by, for example, gently and continuously rocking the cell suspension. Cell suspension s are aliquoted into wells of 6-well tissue culture dishes, placed inside a sealed, humidified isolation chamber, gassed with 5% CO2, 5% O2, and 90% N2 and placed on a rocker … The rocker is housed inside an incubator maintained at 37oC. The culture plates can be rocked continuously for at least 48 hours and up to 14 days" (e.g. column 4, 3rd full paragraph). Rocking suspension culture on a rocker is a type of agitation of the suspension culture. Thus, claims 1-3 and 31 are anticipated by Thomson.

10. Claims 1, 3 and 31 are rejected under 35 U.S.C. 102(a) as being anticipated by Dang et al., June 26, 2003 (US 2003/0119107 A1, IDS).

Claims 1, 3 and 31 are directed to a method for producing embryoid bodies (EBs) from multi- or pluripotent cells, such as embryonic stem cells, comprising agitation of a liquid suspensiion culture of multi- or pluripotent cells in a container until generation of cell aggregates and optionally diluting the suspension and further agitation of the suspension until formation of EBs, and an embryoid body obtained from said method.

Dang teaches a novel bioprocess where aggregation of ES cells and EBs are controlled, and the EBs can be generated with high efficiency and cultured in high cell density and well-mixed system (e.g. abstract). Dang teaches a method for efficient formation of EBs and the culture of EBs in suspension at higher cell densities by controlling cell aggregation via stirring or agitation of the liquid suspension (e.g. [0051], [0053]). Thus, claims 1, 3 and 31 are anticipated by Dang.

***Claim Rejections - 35 USC § 103***

11. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

12. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later

invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

13. Claims 1 and 4-6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson et al., 2003 (US Patent No. 6,602,711 B1) in view of Dang et al., 2002 (Biotechnol Bioeng, Vol. 78, p. 442-453).

Claims 1 and 4-6 are directed to a method for producing embryoid bodies (EBs) from multi- or pluripotent cells, such as embryonic stem cells, comprising agitation of a liquid suspensiion culture of multi- or pluripotent cells in a container until generation of cell aggregates and optionally diluting the suspension and further agitation of the suspension until formation of EBs, and an embryoid body obtained from said method. Claim 4 specifies the cells are derived from a murine ES cell line. Claim 5 specifies the culture medium is IMDM, 20% FCS and 5% CO2. Claim 6 specifies the culture condition comprises 37oC and 95% humidity.

Thomson teaches a method for producing primate embryoid bodies from colonies of primate embryonic stem cells by removing the adhering colonies of the embryonic stem cells from the substrate in clumps and then incubating the clumps in a container under conditions that essentially inhibit the clumps from attaching to the container and under conditions in which the clumps of embryonic stem cells coalesce into embryoid bodies (e.g. claim 1). Thomson teaches culturing primate embryonic stem cells, such as rhesus or human ES cells, on inactivated mouse embryonic fibroblasts with culture medium DMED and 20% FBS and the ES cell colonies are removed from the tissue culture plate using physical or chemical methods, such as dispase or collagenase (e.g. column 3, lines 48-67). Thomson further teaches "[o]nce colonies are removed from the tissue culture plate, the ES cells should remain in suspension during further embyoid

body formation. This can be achieved by, for example, gently and continuously rocking the cell suspension. Cell suspension s are aliquoted into wells of 6-well tissue culture dishes, placed inside a sealed, humidified isolation chamber, gassed with 5% CO2, 5% O2, and 90% N2 and placed on a rocker … The rocker is housed inside an incubator maintained at 37oC. The culture plates can be rocked continuously for at least 48 hours and up to 14 days" (e.g. column 4, 3rd full paragraph). Rocking suspension culture on a rocker is a type of agitation of the suspension culture.

Thomson does not specifically teach using murine ES cells, IMDM culture medium, 20% FCS and 95% humidity.

Dang teaches culturing CCE murine embryonic stem cell in IMDM medium at 37oC in humidified air with 5% CO2 (e.g. p. 444, left column, Materials and Methods). Dang use liquid suspension cultures of murine ES cells to form EBs (e.g. p. 444, right column, 1st paragraph).

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to culture murine ES cells in IMDM medium at 37oC in humidified air with 5% CO2 because Dang teaches culturing murine ES cells in said condition. It also would have been prima facie obvious for one of ordinary skill in the art to culture ES cells in medium having 20% FCS and 95% humidity because Thomson teaches culturing ES cells in medium having 20% FBS and both Thomson and Dang teach culturing ES cells in humidified condition. FBS and FCS are the same and growing ES cells in 95% humidity would be obvious to one of ordinary skill in order to optimize the culture condition for the ES cells. Determining effective dose or conditions for culturing ES cells is routine optimization of a result-effective variable and is obvious to one of ordinary skill.

One having ordinary skill in the art at the time the invention was made would have been motivated to do so in order to form EBs from primate ES cells as taught by Thomson or to form EBs from murine ES cells as taught by Dang with reasonable expectation of success.

14. Claims 1, 7-18, 32 and 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson et al., 2003 (US Patent No. 6,602,711 B1) in view of Kehat et al., 2001 (The Journal of Clinical Investigation, Vol. 108, No. 3, p. 407-414, IDS).

Claims 1, 7-18, 32 and 33 are directed to a method for producing embryoid bodies (EBs) from multi- or pluripotent cells, such as embryonic stem cells, comprising agitation of a liquid suspensiion culture of multi- or pluripotent cells in a container until generation of cell aggregates and optionally diluting the suspension and further agitation of the suspension until formation of EBs, an embryoid body obtained from said method, and a differentiated cell or tissue, which is a cardiomyocyte, derived from the embryoid body. Claim 7 specifies the concentration of multi- or pluripotent cells is about $1x10^6$ to $5x10^6$ cells/ml. Claims 8-10 specify the suspension is cultured for about 6 hours, 16 to 20 hours and in T25 flasks, respectively. Claims 11 and 12 specify the dilution is 1:10 and the final concentration of EBs in the suspension culture is about 500/ml, respectively. Claims 14 and 15 specify the culture of multi- or pluripotent cells has a concentration of $0.1x10^6$ to $0.5x10^6$ cells/ml and the suspension is cultured for about 48 hours, repectively. Claim 16 specifies the EBs are diluted to about 100-2000 EBs/10ml. Claims 17 and 18 specify the cells are differentiated into cardipmyocytes.

Thomson teaches a method for producing primate embryoid bodies from colonies of primate embryonic stem cells by removing the adhering colonies of the embryonic stem cells

from the substrate in clumps and then incubating the clumps in a container under conditions that essentially inhibit the clumps from attaching to the container and under conditions in which the clumps of embryonic stem cells coalesce into embryoid bodies (e.g. claim 1). Thomson teaches culturing primate embryonic stem cells, such as rhesus or human ES cells, on inactivated mouse embryonic fibroblasts with culture medium DMED and 20% FBS and the ES cell colonies are removed from the tissue culture plate using physical or chemical methods, such as dispase or collagenase (e.g. column 3, lines 48-67). Thomson further teaches "[o]nce colonies are removed from the tissue culture plate, the ES cells should remain in suspension during further embyoid body formation. This can be achieved by, for example, gently and continuously rocking the cell suspension. Cell suspension s are aliquoted into wells of 6-well tissue culture dishes, placed inside a sealed, humidified isolation chamber, gassed with 5% CO2, 5% O2, and 90% N2 and placed on a rocker … The rocker is housed inside an incubator maintained at 37oC. The culture plates can be rocked continuously for at least 48 hours and up to 14 days" (e.g. column 4, 3rd full paragraph). Rocking suspension culture on a rocker is a type of agitation of the suspension culture.

Thomson does not specifically teach cell concentration of $1x10^6$ to $5x10^6$ cells/ml or $0.1x10^6$ to $0.5x10^6$ cells/ml, 1:10 dilution, final concentration of EBs in the suspension culture, and culturing the suspension culture for about 6hr or 16-20hr.

Kehat teaches culturing human ES cells in suspension and plated to form EBs, and spontaneously contracting areas appeared in 8.1% of EBs. Cells from said contracting area within EBs were stained positively with anti-cardiac myosin heavy chain, anti-alpha-actinin, anti-desmin, anti-cardiac troponin 1 and anti-ANP antibodies (e.g. abstract). ES cell clumps

were grown in plastic petri dishes at a cell density of about $5x10^6$ cells in a 58 mm dish (e.g. p. 408, bridging left and right column).

It would have been prima facie obvious for one of ordinary skill in the art at the time of the invention to culture the ES cells at a concentration of $1x10^6$ to $5x10^6$ cells/ml or $0.1x10^6$ to $0.5x10^6$ cells/ml, 1:10 dilution, with a certain final concentration of EBs in the suspension culture, and culturing the suspension culture for about 6hr or 16-20hr because Thomson teaches culturing the ES cells for at least 48 hours and up to 14 days and Kehat teaches culturing ES cells at about $5x10^6$ cells in a 58 mm dish. Determining effective dose or conditions for culturing ES cells is routine optimization of a result-effective variable and is obvious to one of ordinary skill. Culturing ES cells at these conditions or with a certain final concentration of EBs would be obvious to one of ordinary skill in order to optimize the culture condition for the ES cells.

One having ordinary skill in the art at the time the invention was made would have been motivated to do so in order to form EBs from primate ES cells as taught by Thomson or to form EBs from human ES cells as taught by Kehat with reasonable expectation of success.

15. Claims 1 and 19-30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson et al., 2003 (US Patent No. 6,602,711 B1) in view of Dang et al., June 26, 2003 (US 2003/0119107 A1, IDS).

Claims 1 and 19-30 are directed to a method for producing embryoid bodies (EBs) from multi- or pluripotent cells, such as embryonic stem cells, comprising agitation of a liquid suspensiion culture of multi- or pluripotent cells in a container until generation of cell aggregates and optionally diluting the suspension and further agitation of the suspension until formation of

EBs, an embryoid body obtained from said method, and a differentiated cell or tissue, which is a cardiomyocyte, derived from the embryoid body. Claims 19-30 specify the cells are genetically engineered using a selectable marker, such as puromycin resistant gene, and/or a reporter gene, such as EGFP, under the control of a cell type-specific regulatory sequence. Claims 27 and 28 specify the marker gene and reporter gene are contained on the same recombinant nucleic acid molecule and on the same cistron, respectively. Claims 29 and 30 specify the cell type-specific regulatory sequence is atrial- and/or ventricular-specific and is selected from promoters of alphaMHC or MLC2v, respectively.

Thomson teaches a method for producing primate embryoid bodies from colonies of primate embryonic stem cells by removing the adhering colonies of the embryonic stem cells from the substrate in clumps and then incubating the clumps in a container under conditions that essentially inhibit the clumps from attaching to the container and under conditions in which the clumps of embryonic stem cells coalesce into embryoid bodies (e.g. claim 1). Thomson teaches culturing primate embryonic stem cells, such as rhesus or human ES cells, on inactivated mouse embryonic fibroblasts with culture medium DMED and 20% FBS and the ES cell colonies are removed from the tissue culture plate using physical or chemical methods, such as dispase or collagenase (e.g. column 3, lines 48-67). Thomson further teaches "[o]nce colonies are removed from the tissue culture plate, the ES cells should remain in suspension during further embyoid body formation. This can be achieved by, for example, gently and continuously rocking the cell suspension. Cell suspension s are aliquoted into wells of 6-well tissue culture dishes, placed inside a sealed, humidified isolation chamber, gassed with 5% CO2, 5% O2, and 90% N2 and placed on a rocker … The rocker is housed inside an incubator maintained at 37oC. The culture

plates can be rocked continuously for at least 48 hours and up to 14 days" (e.g. column 4, 3rd full paragraph). Rocking suspension culture on a rocker is a type of agitation of the suspension culture.

Thomson does not specifically teach using a selectable marker, such as puromycin resistant gene, and/or a reporter gene, such as EGFP, under the control of a cell type-specific regulatory sequence, the marker gene and reporter gene are contained on the same recombinant nucleic acid molecule or on the same cistron.

Dang teaches a novel bioprocess where aggregation of ES cells and EBs are controlled, and the EBs can be generated with high efficiency and cultured in high cell density and well-mixed system (e.g. abstract). Dang teaches a method for efficient formation of EBs and the culture of EBs in suspension at higher cell densities by controlling cell aggregation via stirring or agitation of the liquid suspension (e.g. [0051], [0053]). Dan also teaches individual R1 ES cells expressing GFP protein under the control of a constitutively active promoter was encapsulated with a cyan labeled ES cell, and the two cell types survived and proliferated to form spheroid containing two sources of cells (chimeric spheroids). The technology of forming chimeric spheroids can be used to manipulate the differentiation of the pluripotent cells into specific types of tissue using cell specific signals (e.g. [0185]).

It would have been prima facie obvious for one of ordinary skill in the art at the time of the invention to prepare ES cells expressing a selectable marker, such as puromycin resistant gene, and/or a reporter gene, such as EGFP, under the control of a cell type-specific regulatory sequence because Dang teaches using a ES cell expressing GFP under the control of constitutively active promoter and GFP is a type of selectable marker, and one of ordinary skill

would use another selectable marker or a cell specific promoter in order to optimize the expression of the marker at target cells and to optimize visualization or detection of the ES cells. Having the marker gene and reporter gene contained on the same recombinant nucleic acid molecule or on the same cistron would be obvious to one of ordinary skill because determining effective orientation of the marker gene and reporter gene is routine optimization of a result-effective variable and is obvious to one of ordinary skill. One of ordinary skill would orient the marker gene and reporter gene in a vector in order to optimize the expression of the marker gene and reporter gene at target cells.

One having ordinary skill in the art at the time the invention was made would have been motivated to do so in order to form EBs from primate ES cells as taught by Thomson or to form chimeric spheroids to manipulate the differentiation of the pluripotent cells into specific types of tissue using cell specific signals as taught by Dang with reasonable expectation of success.

It should be noted that since claims 4-33 are improper multiple dependent claims, these claims are interpreted as they all depend from claim 1.

***Priority***

16. Receipt is acknowledged of papers submitted under 35 U.S.C. 119(a)-(d), which papers have been placed of record in the file.

***Conclusion***

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Shin-Lin Chen whose telephone number is (571) 272-0726. The examiner can normally be reached on Monday to Friday from 9:30 am to 6 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272-4517. The fax phone number for this group is (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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